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Crystal Structure and Deletion Analysis show that the Accessory Subunit of Mammalian DNA Polymerase Gamma, PolgB, Functions as a Homodimer

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Introduction: DNA-dependent DNA polymerases are required for the accurate replication and repair of DNA. Replicative DNA polymerases often require accessory factors for highly processive DNA synthesis. Two groups of processivity factors have been described based on their mode of action. One group, known as sliding clamps, are multi-subunit structures that encircle the DNA and slide along it during replication, interacting with the polymerase and preventing dissociation from the DNA. These processivity factors can require additional proteins, known as clamp loaders, for assembly onto the DNA in an ATP-dependent reaction. The second type of processivity factor, typified by thioredoxin as a subunit of T7 DNA polymerase holoenzyme, interacts with the polymerase without the need for ATP hydrolysis or clamp loaders. Some processivity factors are able to interact with DNA in the absence of the polymerase, like the processivity factor of herpes simplex virus, UL42.
Mitochondrial DNA (mtDNA) is replicated and repaired by a nuclear-encoded DNA polymerase, Polg, distinct from the polymerases that replicate and repair nuclear DNA. Polg is composed of two subunits, a catalytic subunit of 125-140 kDa related to the family A of DNA polymerases, and an accessory subunit of 35-51 kDa. The accessory subunit, PolgB, has been characterized as a processivity factor for the polymerase. Upon interaction with the catalytic subunit, PolgB increases the affinity of the polymerase for DNA and promotes tighter nucleotide binding, increasing the polymerization rate.

Results: We have solved the crystal structure of mouse PolgB at a resolution of 1.95 Å. PolgB shows high structural similarity to glycyl-tRNA synthetase and other type IIa aminoacyl-tRNA-synthetases, which like PolgB are homodimers. However, residues critical for ATP-binding and anticodon recognition in tRNA synthetases are not conserved in PolgB. In addition to domains similar to glycyl-tRNA synthetase, PolgB contains a unique intermolecular four-helix bundle that is involved in dimerization. A human PolgB mutant lacking the four-helix bundle failed to dimerize in solution or to stimulate the catalytic subunit PolgA, but retained the ability to bind with PolgA to a primer-template construct, indicating that the functional holoenzyme contains two PolgB molecules. Other mutants retained stimulatory activity, but lost the ability to bind DNA in absence of PolgA. These results suggest that the PolgB dimer contains distinct sites for PolgA-binding, dimerization and DNA-binding.

Conclusions: The structure of the accessory subunit of DNA Polg represents the first structure of a core component of the replication and transcription machinery for mtDNA. The crystallographic data and supporting biochemical evidence clearly show that PolgB is functional as a homodimer and suggest that it associates with one copy of the catalytic subunit in a heterotrimeric holoenzyme. The evolutionary relationship of PolgB to aaRS is reflected in conservation of nucleic acid binding properties, since surface loops involved in tRNA recognition by aaRS appear to be important for the interaction of PolgB with DNA.

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References: Carrodeguas, J.A., et al., Mol Cell Biol, 1999. 19(6): p. 4039-46

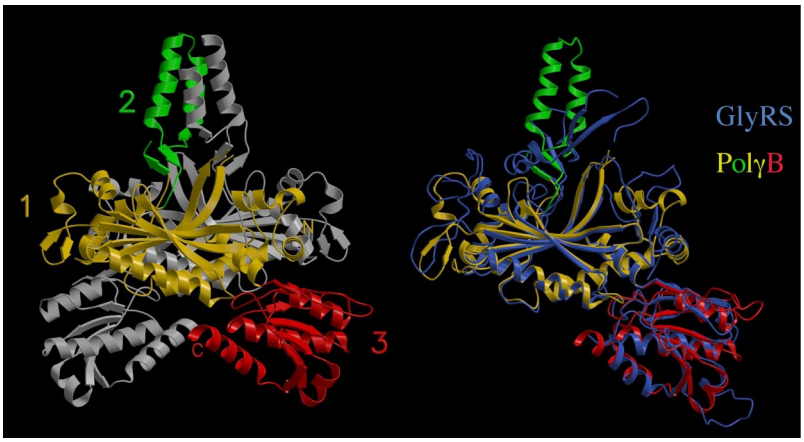

Figure: (left) PolgB forms a dimer (one subunit shown colored, the other in gray) held together by an intermolecular four-helix bundle of domain 2. (right) Two of the three domains of PolgB share high structural similarity with glycyl-tRNA synthetase.